[Chem. Pharm. Bull. 18(10)1951—1959(1970)]

UDC 547.94.02 : 582.675.4.04

Alkaloids of the Leaves of *Cocculus laurifolius* DC. II.[1,2] The Structure of Erythroculine[3]

Yasuo Inubushi,[4a] Hiroshi Furukawa,[4b] and Motoharu Ju-ichi[4a]

Faculty of Pharmaceutical Sciences, Kyoto University[4a]

(Received February 23, 1970)

A new alkaloid designated as erythroculine was isolated from the leaves of *Cocculus laurifolius* DC. (Menispermaceae). From degradative and spectroscopic studies, the structure (I) was assigned to this alkaloid and this assignment was then definitely confirmed by chemical correlation of erythroculine with tetrahydroerysotrine (XXII) of established stereochemistry. From the view point of biosynthesis of erythrina alkaloid, a carbomethoxyl group on a benzene ring is unusual and the isolation of this alkaloid makes the third example of erythrina alkaloid in *Cocculus* species.

In a previous paper,[1] we described the isolation and characterization of a new alkaloid, erythroculine (I) as well as three known alkaloids, L-reticuline (II), magnoflorine (III) and laurifoline (IV) from the leaves of *Cocculus laurifolius* DC. (Menispermaceae).

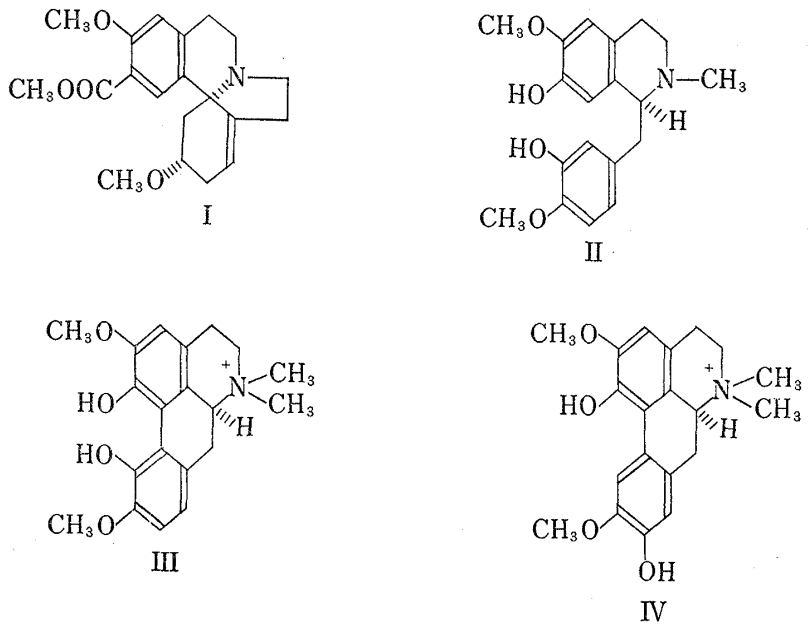

Chart 1

In this paper, we wish to present a detailed account of the structure elucidation and stereochemistry of erythroculine.

Erythroculine resisted all attempts at crystallization but its styphnate crystallized from acetone to yield yellow needles, mp 193—196°. The molecular formula $C_{20}H_{25}O_4N$ was fixed

1) Part I: Y. Inubushi, H. Furukawa, M. Ju-ichi and M. Itoh, *Yakugaku Zasshi*, **90**, 92 (1970).
2) Preliminary communication of this work appeared in *Tetrahedron Letters*, **1969**, 153.
3) This paper constitutes Part CCLVI in the series of "Studies on the Alkaloids of Menispermaceous Plants" by M. Tomita. Part CCLV.[1]
4) Location: *a) Yoshida Shimoadachi-cho, Sakyo-ku, Kyoto*; *b)* Present address: *Faculty of Pharmacy, Meijo University, Yagotourayama Tenpaku-cho, Showa-ku, Nagoya.*

on the basis of elemental analysis of its styphnate. The infrared (IR) spectrum of this base showed the presence of the carbonyl group and the aromatic ring by absorption bands at 1710 cm^{-1} and at 1610, 1570 and 1495 cm^{-1}, respectively. The nuclear magnetic resonance (NMR) spectrum of erythroculine indicated the presence of three methoxyl groups (6.12 τ, 6H, s: 6.71 τ, 3H, s), one tri-substituted double bond (4.37 τ, 1H, m), and two aromatic protons (2.51 τ, 1H, s: 3.29 τ, 1H, s) which are situated in *para* each other. Lack of any signal due to NH and N-methyl group in the NMR spectrum led us to a conclusion that erythroculine must be a tetracyclic alkaloid.

Reduction of erythroculine with lithium aluminum hydride gave erythroculinol (V), $C_{19}H_{25}O_3N$, which showed the hydroxyl band at 3600 cm^{-1} and no carbonyl band in the IR spectrum. In the NMR spectrum of erythroculinol, one of three methoxyl groups in erythroculine disappeared and two-proton singlet due to a hydroxymethylene group at 5.36 τ was newly observed. The hypsochromic shift of the ultraviolet (UV) maxima of erythroculinol (280, 284 mμ: log ε, 3.40, 3.41) compared to that of erythroculine (304 mμ: log ε, 3.62) together with the above mentioned results indicated the presence of a carbomethoxyl group on a benzene ring in erythroculine.

Treatment of erythroculine with boron trichloride in dichloromethane afforded a phenolic compound (VI) in good yield whose IR spectrum revealed a hydroxyl band at 3200 cm^{-1} and a carbonyl band at 1675 cm^{-1}. In the compound (VI), the remarkable bathochromic shift of the carbonyl band compared to that of erythroculine suggested the presence of an intramolecular hydrogen bond between a phenolic hydroxyl group and an ester carbonyl group. These spectral evidences demonstrate that one of two methoxyl groups in erythroculine situates at the *ortho* position to the carbomethoxyl group. Further substitution pattern of the benzene ring was obtained by the following observations. The signals due to two aromatic protons in the NMR spectrum of erythroculine were different in their half-band width and the broader

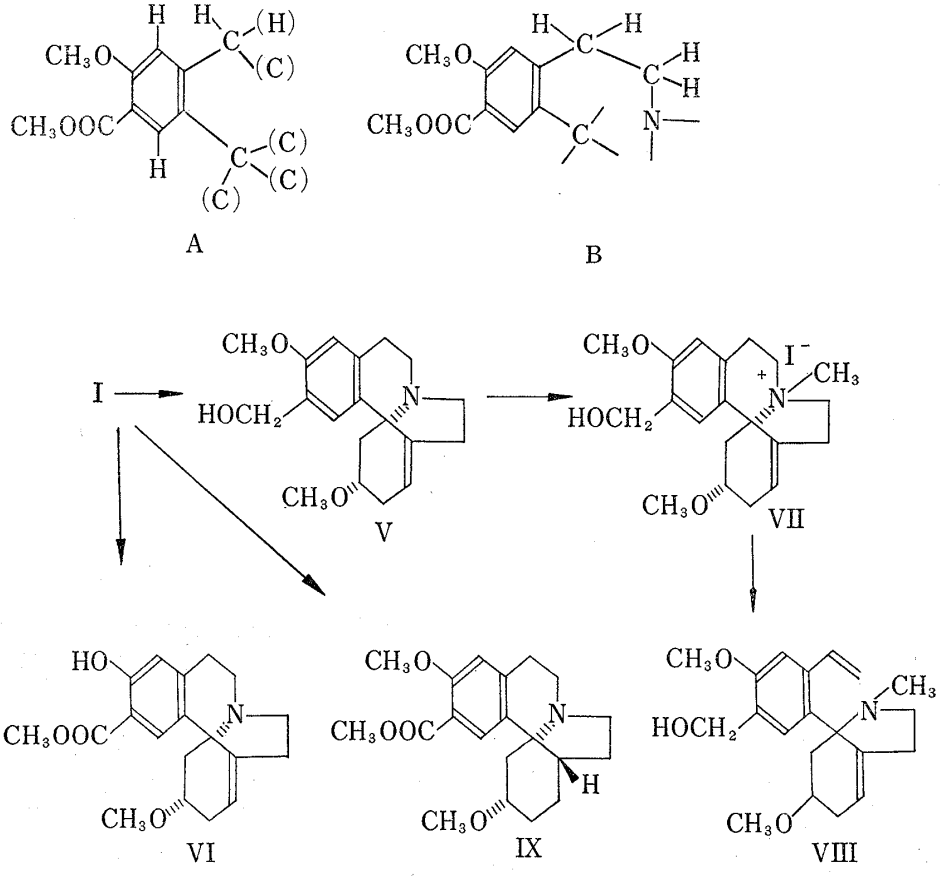

Chart 2

signal at 3.29 τ seems to be coupled with the benzylic proton,[5] while the sharp one at 2.51 τ is not coupled with any proton. The fact that the non-coupled proton signal at 2.51 τ in erythroculine was shifted toward higher field by 0.54 ppm in erythroculinol (V) suggested that this proton is situated at the *ortho* position to the carbomethoxyl group. Moreover, treatment of erythroculinol (V) with D_2O–H_2SO_4[6] which was expected to exchange the *ortho* and/or *para* proton to a methoxyl group with deuterium offered a further information on the situation of two aromatic protons. By this treatment, the relative intensity of the higher field signal (3.29 τ in (I) and 3.39 τ in (V)) to the downfield signal (2.51 τ in (I) and 3.05 τ in (V)) decreased and the latter signal was unaffected. These findings allow us to conclude that the coupled proton at 3.29 τ of erythroculine would be situated at the *ortho* position to a methoxyl group and that erythroculine contains the partial structure shown in (A).

The environment of the nitrogen atom was provided by the Hofmann degradation of erythroculinol (V). Treatment of erythroculinol (V) with methyl iodide afforded erythroculinol methiodide (VII), which was submitted to Hofmann degradation. The methine base (VIII) showed characteristic styrene type absorption in the UV spectrum at 251 and 281 mμ and showed a ABX splitting pattern, each corresponding to one proton at 2.07 τ (1H, q, J=11, 18 cps), 4.50 τ (1H, q, J=2, 18 cps), and 4.77 τ (1H, q, J=11, 2 cps), in the NMR spectrum. Consequently, the partial structure (A) may be expanded to the structure (B).

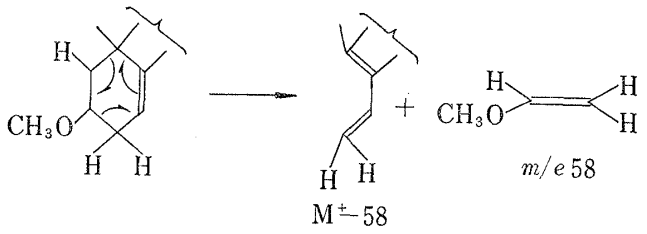

Chart 3

The mass spectrum of erythroculinol (V) confirmed the position of an ethylenic linkage by showing a predominant peak at *m/e* 257 (M^+-58) which generated by a retro Diels–Alder type fragmentation of cyclohexene ring possessing a methoxyl group (see Chart 3).

This fragmentation pattern was known as the diagnostically important one for the aromatic erythrina alkaloids having the cyclohexene ring with a methoxyl group.[7]

The above data led us to the partial structure (C) for another part of erythroculine molecule.

In order to clarify the interrelation between the partial structure (B) and (C), the following experiments were carried out. Hydrogenation of erythroculine over PtO_2 afforded dihydroerythroculine (IX) as a sole product.[8] In the UV spectrum, the compound (IX) and erythroculine showed the absorption maximum at the same wave length (304 mμ) suggesting that tri-substituted double bond is not conjugated with the benzene ring. Acetylation of erythroculinol (V) with Ac_2O–pyridine furnished an acetate (X) which was then submitted to von Braun degradation to give a cyano compound (XI). The compound (XI) showed the characteristic hindered biphenyl absorption in the UV spectrum and revealed two one-proton singlets ascribed to aromatic protons at 2.88 and 3.22 τ together with a methoxyl and an acetyl methyl group at 6.10 and 7.91 τ, respectively, in the NMR spectrum. In addition to these signals, the complex signals corresponding to four aromatic protons between 2.62 and 2.80 τ

5) D.H.R. Barton, R. James, G.W. Kirby, D.W. Turner and D.A. Widdowson, *Chem. Comm.*, **1966**, 294.
6) The description of "D_2O-sodium hydroxide" in a preliminary communication[2] should be read "D_2O-sulfuric acid."
7) *a*) A. Mondon and M. Ehrhardt, *Tetrahedron Letters*, **1966**, 2557; *b*) D.H.R. Barton, R. James, G.W. Kirby, D.W. Turner and D.A. Widdowson, *J. Chem. Soc.*, (*C*), **1968**, 1529; *c*) *Idem.*, *Chem. Comm.*, **1967**, 266.
8) It has already been known that catalytic hydrogenation of this type double bond in the aromatic erythrina alkaloids proceeds stereospecifically. R.K. Hill, "The Alkaloids," Vol. IX, ed. by R.H.F. Manske, Academic Press, New York, 1967, p. 501.

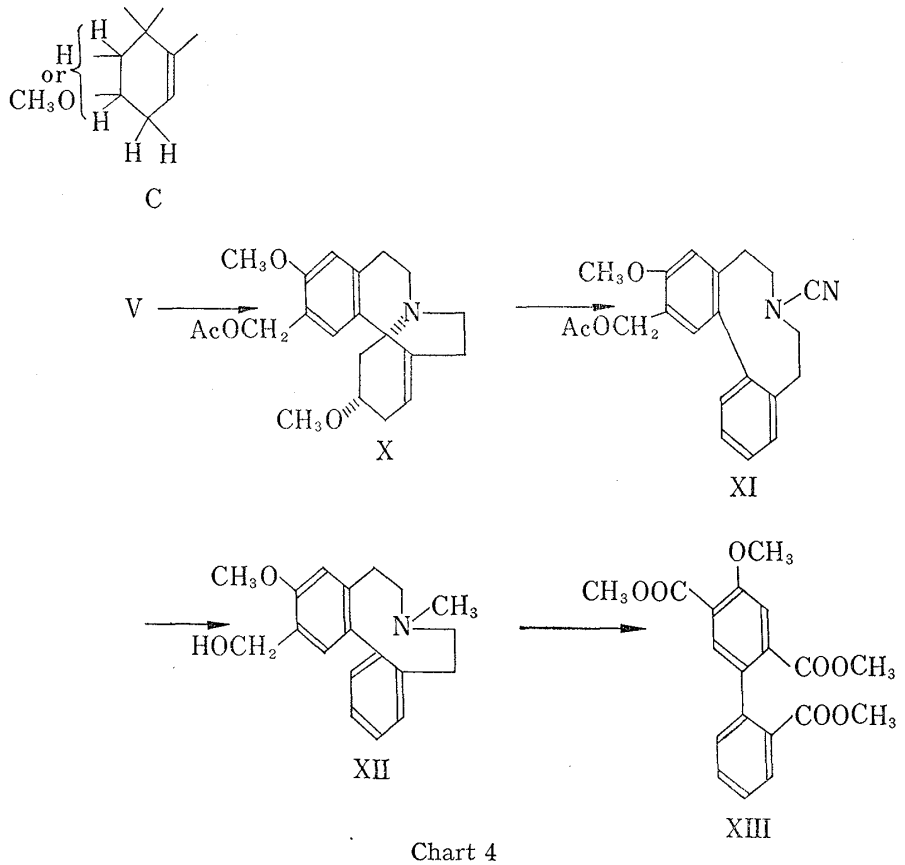

Chart 4

were also observed but one of two methoxyl signals of the compound (X) disappeared. These observations can be well rationalized by supposing that dehydrobromination and elimination of a methoxyl group from the von Braun reaction product occurred to cause aromatization of the cyclohexene ring. It has been well known that this type of reaction is one of the characteristic reactions for the aromatic erythrina alkaloids.[9] Furthermore, the NMR spectrum of this cyano compound (XI) revealed the presence of two A_2B_2 spin systems at 6.58—6.85 τ and 7.25—7.48 τ suggesting the intervention of two ethylene chains between two aromatic rings and the nitrogen atom as shown in the formula (XI).

Reduction of the cyano compound (XI) with lithium aluminum hydride, followed by N-methylation afforded an N-methyl compound (XII) which was then submitted to successive two times Hofmann degradation. The product was then oxidized with potassium permanganate, followed by esterification to furnish a biphenyl (XIII) which was identified with an authentic sample of 4-methoxy-2,5,2′-tricarbomethoxybiphenyl, synthesized through well established route shown below, by thin-layer chromatography (TLC), IR and NMR spectral comparison. Thus, the relative position of the carbomethoxyl group on the benzene ring was established.

The compound (XIV) was prepared from 4-amino-2,5-dimethylanisole[10] by diazotization. Oxidation of this compound with potassium permanganate in pyridine, followed by esterification afforded 4-iodo-2,5-dicarbomethoxyanisole (XV). The Ullmann condensation of (XV) with orthobromoacetophenone (XVI) being catalysed by activated copper bronz was performed in a sealed tube, and the condensation product was hydrolyzed with 5% methanolic sodium hydroxide to give an acidic compound which without purification was esterified. Isolation of (XVII) from the reaction mixture was effected by column chromatography and

9) V. Boekelheide, "The Alkaloids," Vol. VII, ed. by R.H.F. Manske, Academic Press, New York, 1960, p. 213.
10) E. Bamberger, *Ann.*, **424**, 233 (1921).

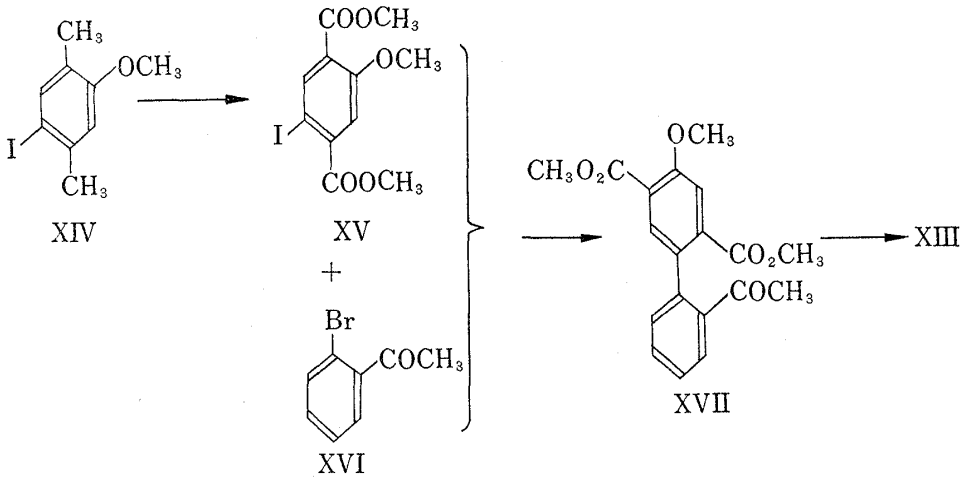

Chart 5

the compound (XVII) was submitted to haloform reaction with aqueous solution of sodium hypochlorite. Esterification of the reaction product afforded the objective compound (XIII).

From all experimental results mentioned so far, erythroculine can be now depicted by the formula (D). The full stereochemistry of erythroculine and the chemical proof of the position of an aliphatic methoxyl group were provided by the correlation of erythroculine with tetrahydroerysotrine (XXII)[11] of established stereochemistry.

Lithium aluminum hydride reduction of dihydroerythroculine (IX) gave dihydroerythroculinol (XIX) which was oxidized with active manganese dioxide to give an aldehyde (XX)

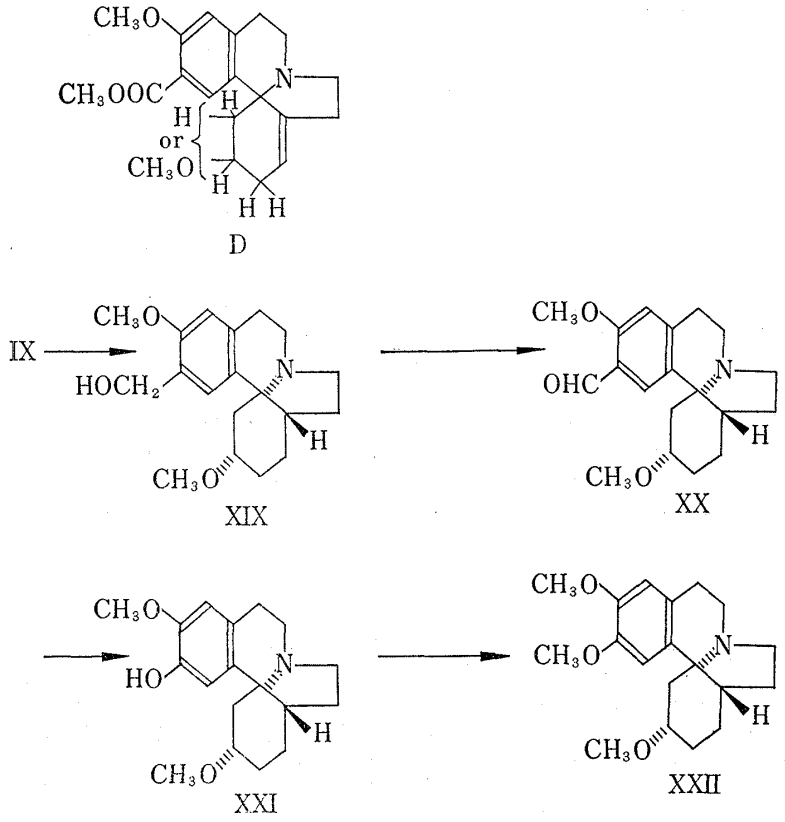

Chart 6

11) G.W. Kenner, H.G. Khorana and V. Prelog, *Helv. Chim. Acta*, **34**, 1969 (1951).

in low yield. On the other hand, treatment of the compound (XIX) with argentic oxide (AgO)[12] in 85% phosphoric acid and acetic acid (1:10) mixture afforded the aldehyde (XX) in good yield, which was submitted to Baeyer–Villiger oxidation with performic acid[13] to give a phenolic base (XXI) in 23% yield. O-Methylation of the product with diazomethane afforded the methyl ether (XXII) which was characterized as its picrate, mp 145—146°, and identified with an authentic sample of tetrahydroerysotrine picrate[14] by comparison of IR (Nujol) spectrum, specific rotation of free base and by admixture melting point of picrate.

Consequently, erythroculine should be represented by the stereostructure of (I).

Erythroculine is the first example of the aromatic erythrina alkaloid possessing a carbomethoxyl group on a benzene ring. From the view point of biogenesis,[7c,15] the origin of the carbomethoxyl group is interesting and the isolation of erythroculine makes the third case[16] of erythrina alkaloids isolated from *Cocculus* species (Menispermaceae).

Experimental[17]

Erythroculine[18] **(I)**——The erythroculine styphnate was recrystallized from acetone to give yellow needles, mp 193—196°. Free base: oil, $[\alpha]_D^{25}$ +194° (c=2.02, $CHCl_3$). UV λ_{max}^{EtOH} mμ (log ε): 304 (3.62), IR ν_{max} cm^{-1}: 1710 (C=O), 1610, 1570, 1495 (aromatic ring). NMR τ: 2.51, 3.29 (each 1H, s, aromatic proton), 4.37 (1H, m, olefinic proton), 6.12 (6H, s, OCH_3), 6.71 (3H, s, OCH_3). *Anal.* Calcd. for $C_{20}H_{25}O_4N \cdot C_6H_3O_8N_3$: C, 53.06; H, 4.80; N, 9.52. Found: C, 52.86; H, 4.85; N, 9.23.

Erythroculinol (V)——To a suspension of erythroculine (I, 90 mg) in dry ether (10 ml) was added 200 mg of lithium aluminum hydride and the solution was refluxed for 3 hr. Then, the excess of lithium aluminum hydride was decomposed with a few drops of water and extracted with ether. The ethereal extract was dried over anhyd. Na_2SO_4 and evaporated to afford a crystalline residue; Yield 85 mg. Recrystallization from acetone gave erythroculinol (V) as colorless cubes, mp 150—152°, $[\alpha]_D^{20}$ +210° (c=1.02, $CHCl_3$). UV λ_{max}^{EtOH} mμ (log ε): 280 (3.40), 284 (3.41). IR ν_{max} cm^{-1}: 3600 (OH). NMR τ: 3.05, 3.39 (each 1H, s, aromatic proton), 4.40 (1H, m, tri-substituted olefinic proton), 5.36 (2H, s, CH_2OH), 6.14 (3H, s, OCH_3), 6.72 (3H, s, OCH_3). Mass Spectrum m/e: 315 (M^+), 284, 257 (M^+-58, base peak), 238, 226. *Anal.* Calcd. for $C_{19}H_{25}O_3N$: C, 72.35; H, 7.99. Found: C, 72.25; H, 7.95.

Demethylation of Erythroculine——Erythroculine (I, 120 mg) was dissolved in dry CH_2Cl_2 (8 ml) and cooled on a dry ice acetone bath. To this solution was added a solution of boron trichloride in CH_2Cl_2 (2 g in 2 ml) and allowed to stand until the temperature of the reaction mixture was raised to room temperature and additional CH_2Cl_2 was then added. The aqueous layer was separated from the organic layer which was washed with water. The aqueous layer and washings were combined, made alkaline with ammonia and extracted with CH_2Cl_2. The extract was dried over anhyd. Na_2SO_4 and evaporated to give an oily compound (VI); Yield 114 mg. IR ν_{max} cm^{-1}: 3200 (broad, OH), 1675 ($COOCH_3$); the presence of intramolecular hydrogen bonding was confirmed by dilution method. NMR τ: −0.58 (1H, broad s, OH), 2.50, 3.25 (each 1H, s, aromatic proton), 4.35 (1H, m, tri-substituted olefinic proton), 6.07 (3H, s, OCH_3), 6.73 (3H, s, OCH_3). Its picrate was recrystallized from acetone to give yellow cubes, mp 205—207°. *Anal.* Calcd. for $C_{19}H_{23}O_4N \cdot C_6H_3O_7N_3$: C, 53.76; H, 4.65. Found: C, 53.69; H, 4.56.

Deuterium Exchange of an Aromatic Proton of Erythroculinol——In a sealed tube, erythroculinol((V), 40 mg), D_2O (2 ml) and one drop of conc. H_2SO_4 were heated in an oil bath (100°). After 200 hr, the reaction mixture was made alkaline with NH_4OH and extracted with ether. Ethereal extract was washed with water, dried over Na_2SO_4 and evaporated to afford a crystalline compound. Recrystallization from acetone gave colorless cubes, mp 149—152°. Yield 15 mg. NMR τ: 3.05 (1H, s, aromatic proton), 3.37 (⅓H, s, aromatic proton), 4.40 (1H, m, olefinic proton), 5.37 (2H, s, CH_2OH), 6.15 (3H, s, OCH_3), 6.72 (3H, s, OCH_3).

12) L. Syper, *Tetrahedron Letters*, **1967**, 4193.
13) E. Profft and G. Rietz, *J. Prakt. Chem.*, **IV, 11**, 94 (1960).
14) We are grateful to Professor V. Prelog for a gift of an authentic sample of tetrahydroerysotrine picrate.
15) D.H.R. Barton and T. Cohen, "Festschrift A. Stoll," Birkhauser, Basel, 1957, p. 117.
16) M. Tomita and H. Yamaguchi, *Chem. Pharm. Bull.* (Tokyo), **4**, 225 (1956); K. Wada, S. Marumo and K. Munakata, *Arg. Biol. Chem.* (Tokyo), **31**, 452 (1967).
17) All melting points were measured on Yanagimoto Micro Melting Point Apparatus and uncorrected. IR were recorded on Hitachi EPI-S Spectrometer in $CHCl_3$. NMR were measured on Varian A-60 Spectrometer in $CDCl_3$ with tetramethylsilane as internal standard and chemical shifts were given in τ values.
18) Isolation and characterization were reported in an earlier paper.[1]

Hofmann Degradation of Erythroculinol——A solution of erythroculinol (V, 75 mg) and CH_3I (3 ml) in acetone (5 ml) was refluxed over a steam bath. After 1 hr, the reaction mixture was concentrated to deposit a crystalline erythroculinol methiodide (VII). Yield 90 mg. Recrystallization from acetone–methanol mixture gave colorless needles, mp 226—227°. $[\alpha]_D^{23}$ +210° (c=0.50, MeOH). *Anal.* Calcd. for $C_{19}H_{25}O_3N \cdot CH_3I$: C, 52.57; H, 6.18. Found: C, 52.77; H, 6.24.

A solution of methiodide (VII, 90 mg) in methanol (5 ml) was stirred with Ag_2O (freshly prepared from 300 mg of $AgNO_3$) at room temperature. After 3 hr, the precipitate was filtered off and the filtrate was evaporated on a steam bath (40—50°) *in vacuo*. The oily residue was heated in an oil bath (140—150°) under reduced pressure (3 mmHg) and foaming was ceased after 15 min. The reaction product was cooled and extracted with $CHCl_3$. Chloroform extract was washed with water and evaporated to give an oily compound which was chromatographed over silica gel. Elution with dichloromethane gave colorless oil. Yield 43 mg (VIII). IR ν_{max} cm^{-1}: 3550 (OH), 1610, 1490 (aromatic ring). NMR τ: 2.95, 3.03 (each 1H, s, aromatic proton), 4.18 (1H, m, tri-substituted olefinic proton), 5.38 (2H, s, CH_2OH), 6.12 (3H, s, OCH_3), 6.88 (3H, s, OCH_3), 7.68 (3H, s, N–CH_3), 2.07 (1H, q, J=11, 18 cps), 4.77 (1H, q, J=11, 2 cps), 4.50 (1H, q, J=2, 18 cps).

Dihydroerythroculine (IX)——Erythroculine (I, 72.8 mg) was dissolved in acetic acid (6 ml) and hydrogenated over platinum dioxide until uptake of hydrogen ceased. Catalyst was then filtered off and the filtrate was evaporated *in vacuo*. The residue was extracted with ether and evaporation of the solvent gave a colorless oil, dihydroerythroculine (IX). Yield 46.9 mg. UV λ_{max}^{EtOH} mμ (log ε): 304 (3.65). IR ν_{max} cm^{-1}: 1710 ($COOCH_3$). NMR τ: 2.26, 3.29 (each 1H, s, aromatic proton), 6.11 (3H, s, OCH_3), 6.13 (3H, s, OCH_3), 6.73 (3H, s, OCH_3).

Erythroculinol Acetate (X)——To a solution of erythroculinol (V, 340 mg) in pyridine (2 ml), acetic anhydride (2 ml) was added and allowed to stand overnight at room temperature. After the solvent was evaporated *in vacuo*, the residue was extracted with $CHCl_3$. Chloroform extract was washed with water and dried over anhyd. Na_2SO_4 and evaporated to give an oily compound. Yield 300 mg, X. TLC one spot. IR ν_{max} cm^{-1}: 1725 (C=O). NMR τ: 3.00, 3.39 (each 1H, s, aromatic proton), 4.40 (1H, m, tri-substituted olefinic proton), 4.90 (2H, s, $AcOCH_2$–), 6.18, 6.73 (each 3H, s, OCH_3), 7.91 (3H, s, CH_3CO).

von Braun Degradation of Erythroculinol Acetate (X)——A solution of erythroculinol acetate (X, 300 mg) in benzene (6 ml) was treated with BrCN (*ca.* 1 g) and allowed to stand overnight at room temperature. The solvent was evaporated *in vacuo* and the residue was dissolved in $CHCl_3$ and the solution was washed with 5% HCl solution, dried over anhyd. Na_2SO_4 and evaporated. The residue was chromatographed over silica gel to give an oily compound (XI). Yield 260 mg. UV λ_{max}^{EtOH} mμ (log ε): 284 (3.46, hindered biphenyl). IR ν_{max} cm^{-1}: 2220 (CN), 1730 (OAc), 1615, 1510 (aromatic ring). NMR τ: 2.62—2.80 (4H, m, aromatic protons), 2.88, 3.22 (each 1H, s, aromatic proton), 4.82 (2H, s, CH_2OAc), 6.10 (3H, s, OCH_3), 6.58—6.85 (4H, m, $-C\underline{H}_2-(CH_2)-N\times 2$), 7.25—7.48 (4H, m, $-C\underline{H}_2-N\times 2$), 7.91 (3H, s, OAc).

Lithium Aluminum Hydride Reduction of Cyano Compound (XI) and N-Methylation——To a solution of the cyano compound (XI, 260 mg) in dry tetrahydrofuran (10 ml) was added a large excess of lithium aluminum hydride (*ca.* 800 mg) and the reaction mixture was refluxed for about 5 hr. After the excess of lithium aluminum hydride was decomposed, the reaction mixture was extracted with ether and the extract was dried over anhyd. Na_2SO_4 and evaporated to give an oily compound. Yield 250 mg. To a solution of the above product, without further purification, in MeOH (10 ml) was added 2 ml of 37% formalin. This mixture was stirred for 1 hr at room temperature, and then $NaBH_4$ (420 mg) was added to the mixture under cooling in an ice-bath. The reaction mixture was stirred for a few minutes at room temperature. The solvent was evaporated *in vacuo* and the residue was extracted with $CHCl_3$. The extract was washed with water, dried over anhyd. Na_2SO_4 and evaporated to give an oily compound (XII). Yield 240 mg. IR ν_{max} cm^{-1}: 3550 (OH). NMR τ: 2.95, 3.26 (each 1H, s, aromatic proton), 2.66—2.88 (4H, m, aromatic protons), 5.32 (2H, s, CH_2OH). 6.10 (3H, s, OCH_3), 7.68 (3H, s, NCH_3). Its picrate was recrystallized from acetone to give yellow cubes, mp 209—212°. *Anal.* Calcd. for $C_{19}H_{23}O_2N \cdot C_6H_3O_7N_3 \cdot \frac{1}{2}$ Acetone: C, 57.30; H, 5.22. Found: C, 56.90; H, 5.51.

Hofmann Degradation of N-Methyl Base (XII) and Potassium Permanganate Oxidation of the Product——To a solution of N-methyl base (XII, 240 mg) in acetone (5 ml) was added CH_3I (2 ml). After standing overnight, the reaction mixture was refluxed on a water bath for 30 min and the solvent was evaporated. The residue was dissolved in methanol (5 ml), and Ag_2O (freshly prepared from $AgNO_3$ 250 mg) was added to this solution, which was stirred for 2 hr at room temperature. Precipitate was filtered off and the filtrate was evaporated *in vacuo*. When the residue was heated in an oil bath under reduced pressure, foaming occurred at 147°/2 mmHg, and this temperature was kept for 15 min. The methine base, showing two spots on TLC, was then converted to its methohydroxide by treatment with the same procedure mentioned above and the second stage Hofmann degradation was carried out at 195°/2 mmHg. After cooling, the residue was extracted with the mixture of MeOH and acetone (1:1) and the extract was evaporated to give the crude des-N compound. To a solution of this crude product in acetone (10 ml) was added 3% potassium permanganate solution in acetone and water (1:1, 60 ml) under stirring at room temperature. Stirring was continued on a water bath (*ca.* 80°) for 2 hr, and the reaction mixture was cooled and made acidic with sulfuric acid. Precipitated MnO_2 was decomposed with $NaHSO_3$ and the solution was concentrated *in vacuo*.

The solution was then saturated with NaCl and extracted with AcOEt. The extract was evaporated and the residue was dissolved in methanol. To this solution was added the ethereal solution (15 ml) of diazomethane (prepared from nitrosomethylurea 3 g as usual) and allowed to stand for 30 min. Excess CH_2N_2 was decomposed with dil. HCl and the solvent was evaporated *in vacuo*. The residue was extracted with ether and the extract was dried over anhyd. Na_2SO_4 and evaporated. Yield 29 mg. Repeated column chromatography over silica gel afforded the homogeneous product (XIII) as a colorless oil. Yield 3 mg. IR ν_{max} cm^{-1}: 1720 (C=O). NMR τ: 1.90—2.90 (6H, m, aromatic protons), 6.00, 6.13, 6.37, 6.40 (each 3H, s, $COOCH_3$). Mass Spectrum *m/e*: 358 (M^+), 327 (M^+-31), 299 (metastable peak of $M^+ \rightarrow M^+-31$). The IR, NMR spectrum and TLC of the product were identical with those of an authentic sample prepared through the undermentioned synthetic route.

4-Iodo-2,5-dicarbomethoxy-anisole (XV)——4-Iodo-2,5-dimethylanisole[19] (XIV, 2 g) prepared from 4-amino-2,5-dimethylanisole[10] by diazotization as usual, was added to a solution of potassium permanganate (7.9 g) in pyridine (25 ml), water (35 ml) and 10% KOH solution (1 ml) with stirring at room temperature. After 1 hr, the reaction mixture was warmed on a water bath (80—85°) for 3 hr. After cooling, the reaction mixture was made acidic with H_2SO_4, and the precipitating MnO_2 was dissolved by addition of sodium bisulfite. This solution was saturated with NaCl and extracted with AcOEt. The extract was evaporated *in vacuo* and the residue was dissolved in MeOH (50 ml) and the solution was saturated with HCl gas and allowed to stand overnight at room temperature and then, refluxed for 1 hr. The solvent was evaporated and the residue was extracted with ether and the extract was dried over $MgSO_4$ and evaporated to give a crystalline mass (XV). Yield 1.1 g. Recrystallization from ether-acetone gave colorless needles, mp 112°. IR ν_{max} cm^{-1}: 1720 ($COOCH_3$). NMR τ: 1.72, 2.63 (each 1H, s, aromatic proton), 6.06 6.10, 6.12 (each 3H, s, OCH_3, $2 \times COOCH_3$). *Anal.* Calcd. for $C_{11}H_{11}O_5I$: C, 37.71; H, 3.14. Found: C, 37.63; H, 3.21.

2′-Acetyl-2,5-dicarbomethoxy-4-methoxybiphenyl (XVII)——4-Iodo-2,5-dicarbomethoxyanisole (XV, 500 mg) and Cu powder (500 mg) was well mixed and *o*-bromoacetophenone (XVI, 591 mg) was added to this mixture. The mixture was placed in a sealed tube and heated in an oil bath (220—230°) for 2 hr. After cooling, the reaction mixture was extracted with a mixture of acetone and MeOH (1:1). The extract was evaporated and the residue was dissolved in 5% NaOH solution (MeOH–H_2O 1:1, 40 ml) and refluxed for 2 hr. Methanol was evaporated *in vacuo* and the resulting aqueous layer was diluted with water and extracted with ether to remove neutral compounds. Alkaline aqueous layer was made acidic with conc. HCl and extracted with AcOEt. The extract was evaporated to give a crystalline compound, which was dissolved in MeOH (30 ml). This solution was saturated with HCl gas and left on standing at room temperature overnight. After refluxing for 2 hr, the solvent was evaporated *in vacuo* to give the residue which was extracted with ether. The extract was successively washed with 5% NaOH, water and dried over $MgSO_4$, and evaporated to give an oily compound. Yield 400 mg. The residue was chromatographed over Al_2O_3 (10×60 mm) and the benzene eluate was collected. Recrystallization from ether gave 2′-acetyl-2,5-dicarbomethoxy-4-methoxybiphenyl (XVII) as colorless cubes. mp 178—180°. Yield 40 mg. IR ν_{max} cm^{-1} 1720 ($COOCH_3$), 1685 ($COCH_3$). NMR τ: 2.14—2.88 (6H, aromatic protons), 6.00, 6.13, 6.36 (each 3H, s, OCH_3, $2 \times COOCH_3$), 7.75 (3H, s, $COCH_3$). Mass Spectrum *m/e*: 342 (M^+), 299 (M^+-43), 261.2 (meta stable peak of $M^+ \rightarrow M^+-43$), 283 (M^+-59), 234.0 (meta stable peak of $M^+ \rightarrow M^+-59$).

Haloform Reaction of the Compound (XVII)——To an aqueous solution of NaOCl (containing 10% Cl, 3 g in 20 ml water) was added an ethereal solution of the Ullmann condensation product (XVII, 115 mg). Then, ether was evaporated by blowing air and the remaining solution was warmed with stirring on a steam bath (60—75°) for 3 hr. After the excess sodium hypochlorite was decomposed by addition of dil. $NaHSO_3$ solution, the reaction mixture was made alkaline with 5% NaOH solution and extracted with ether to remove the neutral compound. The aqueous layer was made acidic with conc. HCl and extracted with AcOEt. The extract was evaporated and the residue was esterified by using methanol–HCl as usual. The product was successively chromatographed over silica gel and alumina. The pure compound was obtained as a colorless oil (XIII). Yield 9 mg. IR ν_{cm}: 1720 (C=O). NMR τ: 6.00, 6.12, 6.36, 6.39 (each 3H, s), 1.90—2.89 (6H, aromatic protons). Mass Spectrum *m/e*: 358 (M^+), 327 (M^+-31), 299 (meta stable peak of $M^+ \rightarrow M^+-31$).

Dihydroerythroculinol (XIX)——To a suspension of dihydroerythroculine (IX, 3.2 g) in 100 ml of dry ether was added lithium aluminum hydride (1 g). The reaction mixture was refluxed for 3 hr, cooled and extracted with ether. The extract was dried over anhyd. Na_2SO_4 and evaporated to yield an oily compound (dihydroerythroculinol (XIX)). Yield 2.37 g. IR ν_{max} cm^{-1}: 3550 (OH). NMR τ: 2.85, 3.40 (each 1H, s, aromatic proton), 5.39 (2H, s, CH_2OH), 6.19, 6.73 (each 3H, s, OCH_3).

Argentic Oxide Oxidation of Dihydroerythroculinol (XIX)——To a solution of dihydroerythroculinol (XIX 1.39 g) in the mixture (100 ml) of AcOH and 85% H_3PO_4 (10:1) was added argentic oxide (1.09 g) with stirring at room temperature. After standing for 2 days, the reaction mixture was diluted with water, made alkaline with NH_4OH, and extracted with ether. Ethereal extract was evaporated to give an oily compound. The solution of the compound in CH_2Cl_2 was chromatographed over silica gel and the acetone

19) R.L. Cohen and A.J. Sisti, *Can. J. Chem.*, **42**, 1388 (1964).

eluate was collected. Yield 1.26 g (XX). IR ν_{max} cm^{-1}: 1670 (C=O), 1605, 1490 (aromatic ring). NMR τ: −0.40 (1H, s, CHO), 2.29, 3.29 (each 1H, s, aromatic proton), 6.11, 6.77 (each 3H, s, OCH_3). The picrate was recrystallized from acetone to give yellow needles, mp 219—220°. *Anal.* Calcd. for $C_{19}H_{25}O_3N \cdot C_6H_3O_7N_3$: C, 55.14; H, 5.18. Found: C, 55.21; H, 5.15.

Baeyer-Villiger Oxidation of the Aldehyde (XX)——To a solution of the aldehyde (XX, 200 mg) in 3 ml of 85% formic acid was added the performic acid solution (2 ml) prepared from 30% H_2O_2 (1.13 g) and 85% formic acid (10 ml) under cooling in an ice-bath and the reaction mixture was allowed to stand at room temperature for 210 hr. Then, a small amount of $Na_2S_2O_3$ was added to the reaction mixture and formic acid was evaporated *in vacuo*. The residue was dissolved in 5% HCl solution and extracted with ether to remove neutral compound. The aqueous solution was made alkaline with NH_4OH and extracted with CH_2Cl_2, and the extract was dried over anhyd. Na_2SO_4 and evaporated. The residue was chromatographed over silica gel and the acetone eluate was collected to give an oily compound (XXI). Yield 44 mg. TLC, one spot. IR ν_{max} cm^{-1}: 3525 (OH). NMR τ: 3.17, 3.41 (each 1H, s, aromatic proton), 5.72 (1H, broad, disappeared by treatment with D_2O), 6.15, 6.74 (each 3H, s, OCH_3).

Tetrahydroerysotrine (XXII)——To a solution of the product (XXI, 44 mg) in MeOH (10 ml) was added a solution of diazomethane, prepared from nitrosomethylurea (3 g), in ether. The mixture was left on standing at room temperature overnight and worked up as usual. The product was purified by chromatography over alumina to give tetrahydroerysotrine (XXII) as an oil. Yield 29.3 mg. The IR spectrum of this compound showed no hydroxyl band. NMR τ: 3.24, 3.40 (each 1H, s, aromatic proton), 6.15, 6.16, 6.73 (each 3H, s, OCH_3). $[\alpha]_D^{25}$ −24.0° (c=0.83, 95% EtOH, lit.[11] −23.3°). The picrate was recrystallized from a mixture of EtOH and acetone to give yellow cubes, mp 145—146° (lit.[11] 153°). *Anal.* Calcd. for $C_{19}H_{27}O_3N \cdot C_6H_3O_7N_3$: C, 54.94; H, 5.53. Found: C, 55.02; H, 5.73. This picrate showed two crystal forms, mp 140—142° and mp 145—146°, respectively and the spectrum of each specimen in Nujol mull was not superimposable with each other. Each specimen of these crystal forms was interchangeable with another form by the cross seeding method. The IR spectrum (Nujol mull) of the picrate of the sample, which was converted from erythroculine, was identical with that of the authentic sample of tetrahydroerystorine picrate (mp 145—146°),[20] and the admixture melting point of two picrates was undepressed.

Acknowledgement The authors wish to thank Emeritus Professor M. Tomita, Kyoto University, for his hearty encouragement. Thanks are also due to Dr. T. Shingu and Miss M. Ohkawa for NMR spectrum measurements, to Mr. A. Kato for mass spectrum measurements and to the members of the Microanalytical Center of this University for elemental analyses.

20) The authentic sample of tetrahydroerysotrine picrate provided by Prof. Prelog melted at 145—146° by our melting point determination apparatus.